AN ASCOBACTERIUM FROM THE SUGAR-CANE, WITH NOTES UPON THE NATURE OF THE SLIME

(Bacterium sacchari, n.sp.)

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(Plate vi.)

During an investigation into the gummosis of the sugar-cane an organism was met with great frequency; in fact, so often was it found that I cannot but regard it as a normal inhabitant of the cane. In nine cases out of ten it was found in tubes of media inoculated in the fields from the gum of diseased plants, and also from the juice of healthy stalks. It was also separated in the laboratory from the tissue of gummed Rappoe, and of healthy Malabar and Tanna canes. The organism was peculiar, inasmuch as under certain conditions it formed asci, which are well defined masses of capsulated bacteria. The conditions under which these were produced included growth upon a solid medium and the presence of a sugar.

The asci were occasionally formed in fluid media, but only when the layer of fluid was shallow, and the bacteria grew as a ring around the junction of the surface with the glass. The solid medium which was most suited to the growth of the bacterium, and for the formation of asci was cane gelatine, which is prepared by dissolving 10 per cent. of gelatine in cane juice, or in strong diffusion liquor, and neutralising the medium to phenolphthalein with dilute potassium hydrate.

The bacterium was grown in the presence of various sugars, and it was found that either dextrose, levulose maltose, or saccharose would serve equally well as a source from which the asci could be formed. Starch and lactose were useless for this purpose. In the presence of a suitable sugar, salts such as calcium chloride, magnesium sulphate, did not show any advantage over potassium phosphate in accelerating the growth in gelatine media.

There are many races of the bacterium, and these may be classified into two groups. The bacteria of one of the groups form a pale yellow growth on gelatine and agar media; they liquefy gelatine slowly, and produce many asci. The organisms of the other group are deep yellow on agar and gelatine; they liquefy gelatine quickly, and produce few asci. The gummy substance of the asci from both groups is identical. On continued cultivation in the laboratory, the yellow rapidly liquefying races become paler yellow or cream-coloured, and, losing the greater part of their liquefying power, they become identical with the first or normal type whose specific characters are given at the end of this paper.

When the growth was scraped from the surface of saccharose-agar and heated with water, a slimy emulsion, like unbeaten white of egg, was obtained; and as I was at that time searching for the gummosis bacterium, this organism seemed to be very promising. But as the slime had to be tested chemically, and its relation to the gum of the sugar-cane investigated, a considerable quantity of the culture with the accompanying asci was necessary. To obtain a sufficiency of material the bacteria were sown upon the surface of a neutral medium contained in large covered vessels. The medium contained peptone 10, saccharose 100, sodium phosphate 2, potassium chloride 5, agar 20, and tap-water 1,000 grms. In about a week at 30° the growth seemed to have reached a maximum, and after soaking in water for about a quarter of an hour the culture, which had become considerably swollen, was easily separated from the agar.

The swollen emulsion was of a deep yellow colour, and had the consistency of unbeaten white of egg. Numerous attempts were

made to break up the asci and obtain a solution of the slimy material. The most successful method was found to consist in heating the emulsion in the autoclave up to three atmospheres pressure. With this treatment the emulsion separated into a viscous solution and a yellow sediment. The slightly opalescent viscous solution was clarified by using small quantities of aluminium hydrate, care being taken to avoid an excess of the hydrate, which coagulated the gummy material of the solution.

The clear solution was viscous, but readily passed through filter paper. On the addition of alcohol a voluminous white curdy precipitate was thrown down. This readily swelled up in water to form a clear gelatinous unfilterable emulsion. On standing for some weeks the sterilised and clear emulsion became slightly turbid, a light flocculent portion separating out, leaving a filterable solution. From this behaviour it would appear that treatment with alcohol alters the outside surface of the floccules to an insoluble modification, so that on subsequent treatment with water each particle of dissolved slime is enclosed in a thin membrane. The addition of alcohol to the gelatinous solution again gave a curdy precipitate, but on repeating the precipitation several times a stage was reached when the addition of alcohol produced no precipitate, and formed an opalescent solution. However, on adding traces of salts—such as common salt—the curdy precipitate was again formed. The aqueous solution was tested with a number of reagents, and the reactions obtained are as follows:—

Reactions of the mucilaginous slime of the asci.

Lead acetate	opalescence; opaque solution on heating
Basic lead acetate	precipitate
Ammoniacal lead acetate	precipitate
Barium hydrate	no precipitate
Calcium hydrate	precipitate
Copper sulphate	no precipitate
Ferric chloride	no ppt. followed by ammonia gave no ppt. of $Fe(OH)_3$
Hydrochloric acid, dil.	opalescence
Alcohol	curdy precipitate in presence of salts

Aluminium hydrate . ..	coagulation
Acetic acid, dil.	no precipitate
,, ,, glacial	precipitate
Acid mercuric nitrate	ppt. soluble in excess; ppt. reddens on heating
Xanthoproteic reaction	positive
Sulphuric & phosphotungstic acids	precipitate
Acetic and tannic acids	opalescence
Acetic acid and pot. ferrocyanide...	strong opalescence
Hydrochloric acid and pot. mercuric iodide	opalescence
Sodium hydrate & copper sulphate	purplish colour with slight precipitate
Strong mineral acids	reddish-brown colour on heating

No reaction with KI_3, $AgNO_3$, KOH, NaOH, $BaCl_2$, picric acid.

These reactions indicate that the gummy matter which forms the substance of the asci has relations with the carbohydrates as well as the proteids, and at first sight might be taken as being a mucin body. The slimy capsule of some bacteria has been said to consist of a substance "related to mucin, or probably identical therewith."*

Lepierre† claims to have obtained a true mucin from cultures of a fluorescent bacterium. Charrin and Desgrez‡ obtained a mucinous body from bouillon cultures of *Bact. pyocyaneum*. This body swelled up with water, and was filterable. It was precipitated by alcohol, acetic acid, the mineral acids, common salt and magnesium sulphate. The acetic acid precipitate was insoluble in excess, but was dissolved by dilute alkalies. According to the authors, these properties showed it to be a compound mucinoid, but as it contained phosphoric acid it was probably accompanied by a nucleoproteid. A nitrogen determination was apparently not made.

Although the gummy substance of the asci forms a viscous solution, and behaves like mucin in some of its reactions, still differences can be found. The mucins are soluble in lime and

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* Lafar, Technical Mycology, 1898, 40.

† Lepierre, Comptes Rendus, 1898 (126), 761.

‡ Charrin and Desgrez, *ibid*, 596.

baryta waters; this is insoluble in lime water. The colour reactions of the albumens, which are generally decided in the case of the included mucins, are not so with this substance. Finally, the nitrogen percentage shows that it is neither a mucin nor an allied substance.

In the estimation of the nitrogen the clear aqueous solution was precipitated with alcohol and redissolved in water several times until an opalescent alcoholic solution was obtained. This was divided into two parts, one of which was precipitated with common salt, the other with barium chloride. The precipitates were then washed with alcohol and dried until they ceased to lose weight. Portions were taken for the ash determination and for the nitrogen, which was estimated by the Kjeldahl process, due allowance being made for the nitrogen in the sulphuric acid, etc., by check tests with pure saccharose.

Portion precipitated by common salt :—

0·1542 grm. gave 0·0128 grm. ash = 8·30%.

The ash consisted of carbonate, phosphate, sulphate, and chloride of soda.

0·2164 grm. required 0·5 c.c. $^{N}/_{2}$ acid to neutralise the ammonia = 1·76% nitrogen in the ash-free substance.

Portion precipitated by barium chloride :—

0·1054 grm. gave 0·0184 grm. ash = 17·47%.

The ash consisted of carbonate, phosphate, sulphate, and chloride of barium.

0·1820 grm. required 0·25 c.c. $^{N}/_{2}$ acid to neutralise the ammonia = 1·16% nitrogen in ash-free substance.

The amount of dry gum in the cultures is very small—a very viscous solution contains very little solid gum, and on this account only a small quantity was available for the determination. But although these results differ from one another, still they are sufficient to show that the nitrogen in the dry and ash-free slime is less than 2%. This indicates that it is far removed from mucin, which contains about 12%, from pseudomucin with about 10%, and from colloid with about 7%. The presence of phosphoric acid

in the ash has no significance because the culture media contained 0·2% of sodium phosphate. On the whole the albuminoid reactions are rather undecided; the two which were undoubtedly obtained were the xanthoproteic and the phosphotungstic; but these tests alone do not give any definite information regarding the nature of the slime.

If the slime were originally pectose, the method of separating it from bacteria by heating under pressure would have converted it to pectin, which gives a precipitate on the addition of barium hydrate; and as the purified slime does not precipitate with barium hydrate, it is evident that it cannot be pectose. Nor can it be any of the pectin bodies. That the slime contains a pentosan is shown by the production of furfural on distillation with hydrochloric acid, sp.g. 1·16, and also on careful heating with strong sulphuric acid. On hydrolysis, with dilute sulphuric acid at three atmospheres pressure, a reducing substance is formed. This, upon prolonged heating on the water bath with phenylhydrazine, gives an osazone consisting of balls of short needle-shaped crystals, which, when recrystallised, have a melting point of 153° C. The product of acid hydrolysis is probably a pentose, and appears to be similar to that obtained by Bendix* on hydrolysing tubercle and other bacteria. The pentose from tubercle bacteria reduced Fehling's solution, and formed an osazone which melted at 153°-155°.

The formation of a pentose shows that the bacterial slime is related to the vegetable gums.

Bacterium sacchari, n.sp.

Shape, etc.—A short rod, *coli*-like, with rounded ends measures 0·6:1-2 μ, and occurs singly in pairs and threads. On solid media containing sugar, and sometimes on the surface of fluid sugary media, it forms asci. The rods are motile, and the flagella when stained by the night-blue method were seen to vary from one (terminal) to nine (peritricial). With the blues the cells stain

* Bendix, Jour. Chem. Soc. 1901, Abs. ii. 206.

feebly; fuchsin, and especially violet, stain well. Staining by Gram's method is negative. No spores are formed.

Relation to oxygen, etc.—The bacterium does not grow anaërobically, and the optimum temperature is 28° C.

2% glucose-gelatine plate.—The colonies are white glistening, slightly raised and irregular. When magnified they are seen to have a dark clouded, convoluted or areolate centre, and a finely granular margin, with a crenate structure near the smooth edge.

Nutrient gelatine plate.—Whitish or cream-coloured, round, raised, glistening colonies. When magnified the surface colonies are finely granular, the subsurface colonies are coarsely granular, and the deep colonies are rounded and opaque.

Nutrient agar plate.—The colonies vary in colour from dirty white to buff-white; they are rounded, flat and glistening; the centre becomes depressed. When magnified the rounded colonies are finely granular, with large granules distributed chiefly round the centre. The older surface colonies are coarsely granular, with a puckered or wrinkled centre. In crowded plates the small, round, coarsely granular colonies may have a marginal ring (halo). The deep colonies are rounded or lenticular with large coarse granules.

Nutrient agar stroke.—The growth is cream-coloured at 37°, yellowish-white at 22°, slightly raised, glistening, and has a lobular margin. The condensed water carries a slight film.

Neutral cane-agar stroke.—A luxuriant, almost transparent, yellowish-white, raised, spreading growth is formed. It has a gelatinous consistency, and the condensed water is slightly viscous.

Neutral cane-gelatine stroke.—An ivory-white or yellowish-white glistening, irregularly raised growth, with an irregular serrated margin, and gummy or gelatinous consistency is produced. The culture slowly gravitates, and forms a thick gummy mass at the base of the slope. Sometimes there is no apparent liquefaction of the medium until the gravitated gummy mass is removed and a pit revealed; at other times the site of the stroke becomes furrowed by a partial liquefaction of the medium.

Neutral cane-gelatine stab.—The needle track shows a filiform growth, with a hemispherical yellowish nail-head. Neither in this nor in a glucose-gelatine shake were gas bubbles produced.

Nutrient gelatine stab.—The growth is filiform, with a thin, flat, white, glistening and spreading nail-head. The nail-head is depressed, but there is no sign of liquefaction within a week with the normal races. After the seventh day the gelatine beneath the nail head either appears consumed or shows a slight crateriform liquefaction.

Potato.—The growth is thin, dry, deep yellow, flat and glistening.

Carrot and turnip.—A slimy, translucent, whitish growth rapidly spreads over the surface.

Sugar-cane. —A yellow, glistening, gummy growth spreads over the surface, and growing down the vessels exudes at the lower ends as raised globules similar in appearance to the exuded gum of gummy cane.

Sweet wort.—The growth is luxuriant.

Bouillon.—The medium becomes turbid, and forms a film and sediment. A faint indol reaction is sometimes obtained.

Nitrate bouillon. —Nitrate is reduced to nitrite.

Milk.—The medium is coagulated about the tenth day at 28° C., and the reaction is faintly acid.

The ascobacteria which have been described are few in number. *Bact. luteum* (List.), Adametz,* is non-motile, and produces asci in the absence of sugar. The colour of the gelatine cultures is decidedly yellow, and it does not appear to liquefy gelatine. *Bac. citreus*, Unna,* grows very slowly in gelatine, and produces asci in sugar-free media. *Ascobacillus aquatilis*, Moreno,† is stained by Gram's method, and grows quickly at 37°. Like the others the asci are formed in sugar-free media. Since this organism forms asci only in the presence of sugar, and otherwise differs from the bacteria hitherto described, I have named it *Bacterium sacchari.*

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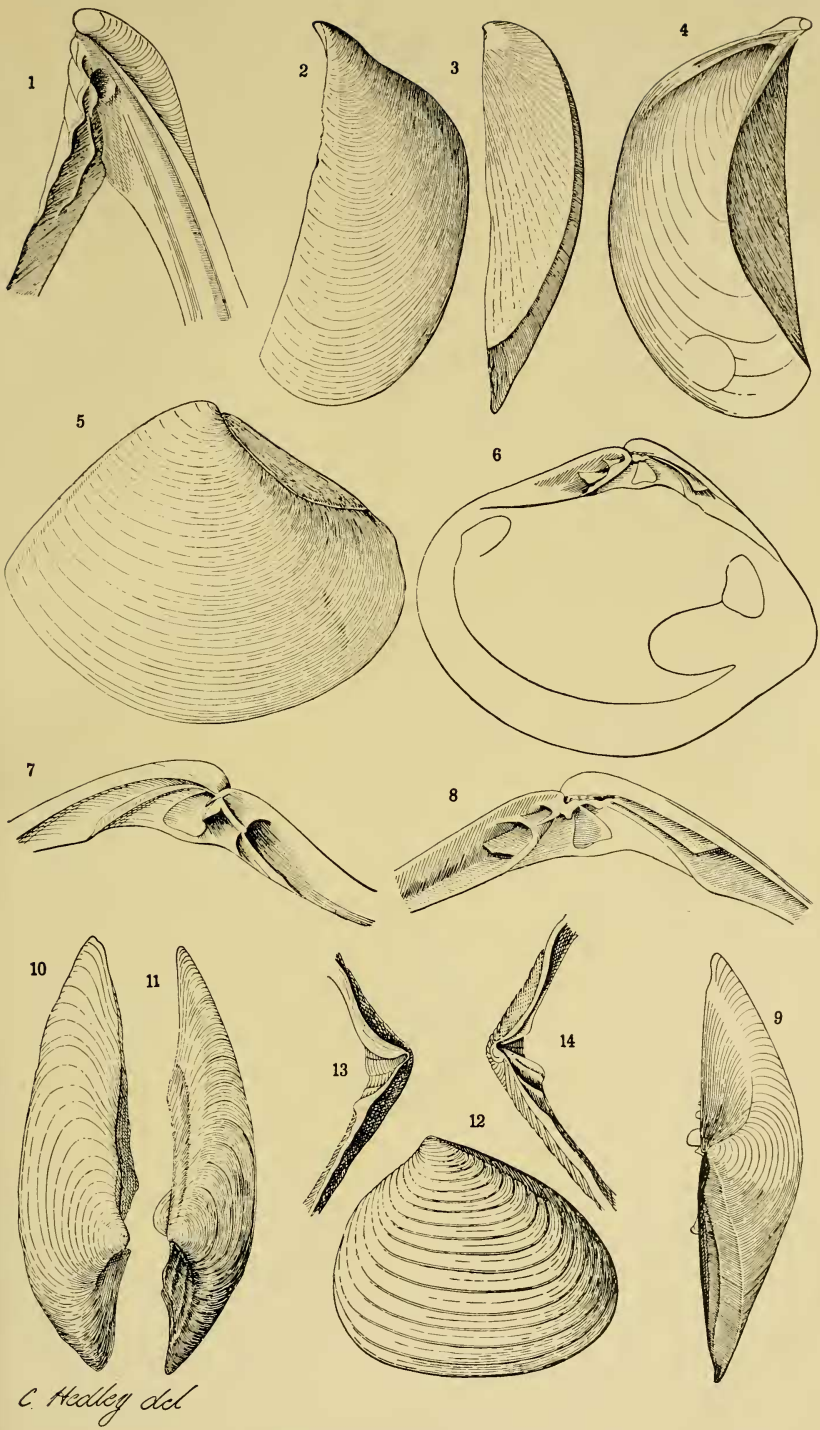
* Migula, System der Bakt. 1900.
† Moreno, Cent. f. Bakt. 1 Abt. 30, 111.



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